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Seed Mycobiota of *Plumbago zeylanica*, Seed Transmission and its Control

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Abstract: ***Plumbago zeylanica is an important medicinal herb used extensively in ethnomedicine. Studies were conducted to determine the seed mycobiota of P. zeylanica. The seedborne nature and seed transmission of the dominant fungus and its chemical management was studied. Present work indicated that rich mycobiota comprising of 14 fungal species of 10 genera were recorded. The dominant fungal species causing leaf blight disease. It is seedborne and seed transmitted and could be managed by seed treatment with Bavistin or Captra.***

Key words: *Plumbago zeylanica, Seed mycoflora,* Fungicides.

1. Introduction

Plumbago zeylanica L., (Plumbaginacae) is an important wild medicinal plant found distributed in the Western peninsula and Bengal. *Plumbago zeylanica* is one among the endangered species (Seetharam et al 1998) and plant contain plumbagic acid and plumbagin, with expectorant and antimicrobial (Ahamad et al 2000; Dhale 2011), anti-inflammatory (Kantha et al 2010), analgesic (Vineet et al 2010), antitussive and anticancer activities (Dhur 1999). The plant species is also important for its traditional medicinal value (Rajakumar and Shivanna 2010). A literature survey suggested that there are no reports of the seed mycoflora of *P. zeylanica* in wild or under cultivation. However, a preliminary study indicated that it is affected by foliar disease incited by *Fusarium chlamydosporum*, through different seasons in the Bhadra Wildlife sanctuary (Parashurama *et al.*, 2013). In view of the above, document the seed mycobiota of this plant species. Hence, the present study was taken up for a detailed study seed mycoflora of *P. zeylanica* and seed transmission with fungicide treatment.

2. Materials and Methods

Field surveys were taken up during 2006-2009 in the study area- Bhadra Wildlife sanctuary (13^0 34^1 to 13^0 46^1 N lat. and 75^0 29^1 to 75^0 45^1 E lon.) of the Western Ghats region of Karnataka. *Plumbago* seeds were collected from the in three forest regions located in the Bhadra Wildlife sanctuary. Seeds from the same region were mixed to obtain three samples. Since the sample size from Lakkavalli and Kakanahosudi forests of Lakkavalli range were small, they were mixed to obtain a single sample. Four hundred seeds of *P. zeylanica* (each from Lakkavalli and Kemmangundi ranges) were incubated by blotter method (Anon 2003). Twenty five seeds were placed in 9 cm dia Petri dishes and incubated under 12 hour of alternating cycles of day/night under fluorescent day light at 22 ± 2^oC for 7 days. Fungi appearing on incubated seeds were identified on the basis of morphological characteristics, fruiting body and reproductive propagules by referring to identification manuals (Barnett et al 1998; Sivanesan 1983 and Subramaniyan 1983). The fungal identity was confirmed by visiting *Index Fungorum* (www.indexfungorum.org). Data of fungal incidence was collected and analyzed statistically.

Seed localization and transmission of the foliar disease causing pathogen. Seeds were subjected to component plating by the technique of Maden et al (1975) to determine the localization of dominant fungal species. The seeds were dissected into its components like the seed coat, cotyledon and embryonic axis, which were surface disinfected and incubated by blotter method at room temperature.

Seed to seedling transmission of the pathogen. Seed transmission of the major fungal species was conducted by the sand method (Karuna and Kolte 2005). Seeds were disinfected as described previously, blotted to remove excess water and artificially inoculated with the homogenized mycelial suspension of the pathogen. Naturally infected seeds (100 seeds) were sown in polypots (10×15 cm) containing the autoclaved potting soil (farmyard manure: soil: sand, 2:1:1) in the green house and irrigated with sterile distilled water. Plants were grown for a period of six weeks. Data on the seedling mortality and disease occurrence in plants were collected. Seedlings were studied for disease symptoms and diseased seedlings were incubated for confirmation of the pathogen involvement. Dead and ungerminated seeds were dug out and analyzed for the pathogen presence. The data of seedling mortality and disease incidence and severity were subjected to analysis of variance (ANOVA) at 5% level of probability (Steel and Torrie 1980).

Management of seedborne fungi. Seed samples of *P. zeylanica* were subjected to fungicide treatment by dusting with Bavistin, Mancozeb, Antracol or Captra @ 2%. The treated seed samples were incubated by blotter method as described previously. The plates were arranged in RCB design with four replications. The data of fungal colonies occurring on treated seed samples as well as seed germination were collected and subjected to ANOVA.

3. Results

Analysis of variance for seedborne occurrence of fungi of *P. zeylanica* revealed that the individual effects for fungi and

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locations used in fungal recovery were significant. Two way interaction of sampling locations and fungi recovered was also significant. A rich mycoflora comprising of 14 fungal species of 10 genera were recorded (Table 1). The incidence of seedborne fungal species varied – *Alternaria alternata* (01-10%), *A. candida* (9.08%), *A. ochraceus* (12.35%), *Aspergillus flavus* (17.27%), *A. niger* (9.69%), *Aspergillus versicolor* (7.04%), *Cladosporium cladosporioides* (27.92%), *F. chlamydosporum* (30.65%), *Pestalotia macrotrichia* (11.35%) and *Rhizopus stolonifer* (3.89%) and species of *Cercospora* (4.08%), *Pencillium* (8.43%), *Periconia* (3.43) and *Phoma* (17.04%) (Table 1). The average percentage of seed germination was 72.69.

Seed localization and transmission. The dominant fungi *Fusarium chlamydosporum* was found to be localized in the seed coat (14.75%), cotyledons (10.50%) and embryo axes (2%); it sporulated profusely in the seed coat region. The naturally infected seeds had germination ability of 73% in pot experiment. The seed sample showed 27% pre-emergence and 8% post-emergence mortalities. Fifteen per cent of seedlings exhibited disease symptoms at 21 days. The pathogen produced symptoms of disease in the form of small lesions on cotyledon and leaf.

Fungicide seed treatment. All the seed dressing fungicides used in the study inhibited fungal occurrence on seeds. Bavistin was highly effective in reducing the occurrence of *F. chlamydosporum* as well as other fungi and improved germination ability of seeds (84.50%) in comparison to control (66.75%). Bavistin is followed by Captra, Hyzeb and Antrocol in their effectiveness (Table 2). Bavistin and Hyzeb completely eliminated the incidence of *A. niger*, *P. macrotrichia*, *A. versicolor* and *R. stolonifer* and significantly reduced *A. alternata* incidence. The seed borne incidence of *A. alternata* was also completely reduced by Hyzeb followed by Captra and Antracol.

4. Discussion

The fungal incidence in two seed samples varied, with high incidence noticed in Lakkavalli sample. Among the fungal species, *F. chlamydosporum* occurred in high incidence in both samples. This fungus also occurred in high percentage in segments of infected foliages in the field and severe damaged to the *Plumbago zeylanica* plants (Parashurama *et al.*, 2013). This suggested that the foliar pathogen *F. chlamydosporum* could become seedborne. The reduced seed germinability in Lakkavalli sample, than in Kemmannugundi sample, could be due to high seedborne infection, particularly by *F. chlamydosporum*. This pathogen is also reported to be seedborne in many plant species including sesame (Dubey 2000; Eman et al 2012). The pathogen is localized in the seed coat as well as in cotyledon and to some extent, in the embryo axis. Since *F. chlamydosporum* could damage cotyledon and radicle, seeds expressed more of the pre-emergence than post-emergence mortality. Observations of the present study suggested that the pathogen is transmitted from mother plant to seed and from seed to seedling. The seed transmission ability of the pathogen could pose a serious challenge during the cultivation of *P. zeylanica* from seeds collected from natural populations in forest regions. Sharfun-Nahar and Mushtaq (2006) also reported that *F. chlamydosporum* is a severe pathogen causing seedborne disease. Similar observation was made on the seed transmission of fungal pathogens in certain medicinal plants (Mallikarjunaswamy 2008).

Among the four fungicides tested for their efficacy, Bavistin was the most effective in reducing the incidence of *F. chlamydosporum* as well as other seedborne fungi. Bavistin, a systematic fungicide, has been used to control seedborne infections caused by species of *Fusarium* (Singh et al 2003; Rajesh and Patel 2011). Other than these fungicides, Captra and Mancozeb were also effective. These fungicides are generally used as foliar spray to manage a variety of fungal diseases. Medicinal plants are not sprayed with any fungicide, even if the disease(s) assumes a high severity. However, seed treatment of medicinal plants with fungicides offers a great advantage since the pathogen inoculum could be managed efficiently, at the earliest with a low level of fungicide. The other advantage could be avoiding the chances of seed transmission of the pathogen to seedlings and transfer of pathogen inoculum to new areas of cultivation.

The fungal pathogens are known to produce mycotoxin in their host systems (Anthony et al 2009) and cause health hazards to humans and veterinary animals when infected plants are consumed either for their food or medicinal value. In the present study, *F. chlamydosporum* has been not been studied for toxin production, however it is known to produce toxins during pathogenisis or in culture (Bosch and Mirocha 1992; Miller and Trenholm 1994). In this regard, the consumption of *Fusarium* infected *P. zeylanica* might pose health hazards to humans in the form of crude medicinal drugs.

The major fungus, *F. chlamydosporum* is seedborne and seed transmitted and caused changes in secondary metabolite content. Further, the major mycobiota could be managed at the seed stage itself by seed treatment with potential fungicides. This will help in the restricting of transportation of potential plant pathogens to newer areas of cultivation, where the pathogen is either not present or is occurring in limited proportion.

5. Acknowledgements

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Table 1: Incidence of mycoflora of *P. zeylanica* seeds collected from forests in sanctuary

Fungal species	*Fungal incidence (%)*		*±SEM*	*CD @ 5%*	*CV (%)*
	Lakkavalli	*Kemmannugundi*			
Alternaria alternata	11.0	6.85	0.47	1.44	18.82
Aspergillus candidus	10.54	7.62	0.49	1.51	19.47
A. flavus	21.85	12.69	0.85	2.62	17.76
A. niger	10.15	9.23	0.51	1.57	18.95
A. ochraceus	14.46	10.23	0.56	1.73	16.39
A. versicolor	7.69	6.38	0.36	1.11	18.52
Cladosporium cladosporioides	30.92	24.92	1.48	4.56	19.09
Cercospora sp.	5.62	2.54	0.19	0.58	16.55
Fusarium chlamydosporum	36.08	25.23	1.77	3.86	14.74
Pestalotia macrotrichia	12.92	9.77	0.56	1.72	17.78
Penicillium sp.	13.23	3.62	0.43	1.32	18.31
Periconia sp.	5.23	1.62	0.19	0.58	19.85

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Phoma sp.	25.77	8.31	0.89	2.73	18.76
Rhizopus stolonifer	4.85	2.92	0.15	0.46	13.83
Germination	66.15	79.23	1.36	4.18	6.72

*Data based on 400 seeds (Each sample in 4 replicate), SBM: Standard Error of mean.

Table 2: Efficacy of seed dressing fungicides on mycoflora of *Plumbago zeylanica* seeds collected from forests in the sanctuary

Fungal species	*Treatments*					*±SEM*	*CD @ 5%*	*CV (%)*
	Control	*Bavistin*	*Hyzeb*	*Antracol*	*Captra*			
Alternaria alternata	10.75	4.50	5.50	2.75	0.0	0.37	1.13	15.66
Aspergillus candidus	13.25	2.0	2.50	1.50	1.0	0.31	0.95	15.29
A. flavus	21.50	1.0	0.0	3.50	3.0	0.43	1.32	14.76
A. niger	13.75	0.0	0.0	0.0	3.50	0.26	0.80	14.97
A. ochraceus	12.75	0.75	1.00	3.50	3.75	0.38	1.19	17.68
A. versicolor	10.0	2.25	2.50	1.75	3.75	0.27	0.83	13.33
Cladosporium cladosporioides	82.0	8.0	4.75	5.75	5.75	1.48	4.56	13.94
Cercospora sp.	15.50	0.50	2.25	1.25	2.50	0.37	1.13	16.73
Fusarium chlamydosporum	31.50	0.75	4.25	7.25	4.0	0.77	2.38	16.19
Pestalotia macrotrichia	25.50	0.00	0.25	0.00	0.00	0.46	1.41	17.73
Penicillium sp.	13.00	0.0	0.0	0.25	0.0	0.21	0.66	16.16
Periconia sp.	13.00	0.0	0.25	0.0	0.0	0.19	0.60	14.62
Phoma sp.	45.75	3.75	5.0	0.0	5.75	0.82	2.54	13.66
Rhizopus stolonifer	10.75	2.25	0.50	0.0	4.75	0.33	1.0	17.86
Germination (%)	66.75	84.50	73.50	70.75	74.50	1.89	5.82	5.11

*Data based on 400 seeds (Each sample in 4 replicate); SEM: Standard Error of mean.

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